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Bovine Pepsinogens and Pepsins. III. Composition and Specificity of the Pepsins*

Helga M. Lang, S. S. J.,† and Beatrice Kassell‡

ABSTRACT: This investigation is concerned with the properties of several pepsins derived from bovine fundic mucosa. The bovine pepsins do not appear to differ among themselves in their amino acid composition, but differences previously found in organic phosphate content are confirmed. Bovine pepsins show similarity in composition to porcine and human pepsins with respect to the large number of acidic and small number of basic residues. However, the content of individual amino acids varies considerably among these pepsins. Bovine pepsins, like human pepsin, have no lysine.

The bovine pepsins do not differ from each other in activity. Comparative studies with porcine pepsin showed rather marked quantitative differences. Both species have similar pH optima. Bovine pepsins have about 25 and 40% of the activity of porcine pepsin toward *N*-acetyl-L-phenylalanyl-L-diiodotyrosine and *N*-acetyl-L-phenylalanyl-L-tyrosine, respectively. Bovine pepsins showed very little activity toward benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-tryptophan ethyl ester, a good substrate for porcine pepsin. Bovine pepsins have 60–70% of the activity of porcine pepsin with hemoglobin as substrate. Bovine and porcine pepsins have similar milk clotting action. The phosphate content has no effect on the activity of the bovine pepsins on any of the substrates tested.

Bovine pepsin was isolated from gastric juice and crystallized by Northrop (1933). Bovine pepsinogen was isolated by Chow and Kassell (1968). Previous studies from this laboratory have demonstrated the large number of differences in amino acid composition between bovine and porcine pepsinogen (Chow and Kassell, 1968) and the constancy of composition, except for changes in organic phosphate, among the major and minor bovine pepsinogens (Meitner and Kassell, 1970). The present report extends these studies to the bovine pepsins prepared from purified pepsinogen. It includes the composition of the major and minor pepsins and their action on proteins and synthetic substrates.

Experimental Section

Substrates. *N*-Acetyl-L-phenylalanyl-L-diiodotyrosine, *N*-acetyl-L-phenylalanyl-L-tyrosine, and benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-tryptophan ethyl ester were products of Cyclo Chemical Corp., Los Angeles, Calif., and were reported by the manufacturer to be chemically pure by chromatography in three different solvent systems.

Enzymes. Crystalline porcine pepsinogen (lot PG 117) was obtained from Worthington Biochemical Corp., Freehold, N. J. Porcine pepsin used for comparative studies was prepared by activation of crystalline porcine pepsinogen in a manner similar to that of Rajagopalan *et al.* (1966).

Activation of Bovine Pepsinogen and Chromatographic Separation of the Pepsins on Hydroxylapatite. Bovine pepsinogen was purified according to the method of Chow and Kassell

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TABLE I: Amino Acid Composition of Bovine Pepsin 1, 2, and 4.

Amino Acid	Residues/Molecule 1	2	4	Av to Nearest Integer	Bovine Pepsinogen[a]	Activation Peptides by Difference
Lysine[b]	0.16 ± 0.17	0.16 ± 0.07	0.23 ± 0.15	0	8	8
Histidine[b]	0.99 ± 0.06	1.02 ± 0.05	1.00 ± 0.04	1	2	1
Arginine[b]	3.02 ± 0.12	2.97 ± 0.08	2.94 ± 0.01	3	6	3
Aspartic[b]	35.00 ± 1.30	35.71 ± 0.66	35.60 ± 0.14	36	40	4
Threonine[c]	24.33 ± 0.90	24.76 ± 1.36	24.13 ± 0.85	25	27	2
Serine[c]	43.42 ± 4.60	45.71 ± 4.74	42.00 ± 2.78	44	50	6
Glutamic[b]	28.08 ± 0.13	28.14 ± 0.35	28.29 ± 0.42	28	32	4
Proline[b]	14.88 ± 0.37	14.51 ± 0.40	14.50 ± 0.29	15	15	0
Glycine[b]	33.51 ± 0.50	33.29 ± 0.41	33.43 ± 0.11	33	35	2
Alanine[b]	14.08 ± 0.20	13.98 ± 0.18	13.93 ± 0.08	14	16	2
Half-cystine[d]	6.08 ± 0.33	6.00 ± 0.34	6.17 ± 0.19	6	6	0
Valine[b]	22.53 ± 0.76	22.28 ± 0.62	22.74 ± 0.68	23	25	2
Methionine[b]	2.78 ± 0.04	2.67 ± 0.12	2.80 ± 0.05	3	4	1
Isoleucine[e,f]	27.89 ± 0.36	27.86 ± 0.25	28.30 ± 0.30	28	32	4
Leucine[b]	19.15 ± 0.22	19.15 ± 0.26	19.06 ± 0.08	19	25	6
Tyrosine[c]	16.13 ± 0.20	16.39 ± 0.34	16.14 ± 0.18	16	18	2
Phenylalanine[g]	14.00	14.00	14.00	14	15	1
Tryptophan[h]	5.20	5.02	4.95	5	6	1
Amide groups[c]	38.26 ± 4.54	29.00 ± 6.56	30.40 ± 5.17	33	37	
Total residues	311	314	310	313	362	49
Molecular weight				33,367	38,943	5576
Organic phosphate	0.53 ± 0.06	1.68 ± 0.12	2.19 ± 0.30			
Specific activity[i]	567	532	543			
$A_{280}^{1\%}$	14.85	14.85	14.73			

[a] Chow and Kassell (1968). [b] Average of 24-, 48-, and 72-hr hydrolysates. [c] Intercept of a straight line calculated by the method of least squares. [d] Determined as cysteic acid according to the method of Moore (1963). [e] Seventy-two-hour hydrolysate. [f] Includes alloisoleucine. [g] Phenylalanine was taken as 14 residues to calculate the residues of amino acid per mole. [h] Tryptophan was determined spectrophotometrically (Edelhoch, 1967). [i] The specific activity is expressed as μg-equiv of Worthington crystalline porcine pepsin/A_{280} unit of bovine pepsin (Chow and Kassell, 1968).

(1968) and was activated by the method of Kassell and Meitner (1970). Following the separation of the activation peptides on Amberlite IRC-50, chromatography of the active pepsin on hydroxylapatite (Figure 1) separated the enzyme into fractions designated 1, 2, and 4. These correspond to fractions 1, 2A, and 4 in our previous paper (Kassell and Meitner, 1970). The tail portion of peak 2 did not differ from the main portion in activity, amino acid composition, or phosphate content. Previous studies have shown that the relative amounts of these fractions differ in various preparations. In this case, peak 3 was not obtained in a significant amount. Each pepsin peak showed uniform activity across the peak (hemoglobin assay at pH 2) and the specific activity for each fraction was the same within experimental error (Table I). Contaminating inorganic phosphate was removed by extensive dialysis as described previously (Meitner and Kassell, 1971).

Enzyme Assay. The proteinase activity of pepsin was determined by a modification of the hemoglobin method of Anson (1939) and milk clotting activity was determined by a modification of the procedure of Seijffers *et al.* (1963); both modifications were described previously (Chow and Kassell, 1968). The enzyme concentration was determined spectrophotometrically at 280 nm using a factor of 676 μg of protein/A_{280}. The value for porcine pepsin was known (Blumenfeld *et al.*, 1960); the same factor was found in the present studies for bovine pepsin. Molecular weights of 34,163 for porcine (Rajagopalan *et al.*, 1966) and 33,367 for bovine pepsin, determined from amino acid analyses, were used to calculate molar concentrations.

Amino Acid Analyses. Amino acid analyses of the bovine pepsins were performed by the procedure of Moore and Stein (1963), using β-2-thienylalanine (Siegel and Roach, 1961) and α-amino-β-guanidopropionic acid (Walsh and Brown, 1962) as internal standards for the long and short columns, respectively. Approximately 15-mg samples of protein were dissolved in 10 ml of water from which 1-ml samples were taken for hydrolysis. The water was evaporated in the hydrolysis tubes *in vacuo* and the residue was hydrolyzed in redistilled 5.7 N HCl for 24, 48, and 72 hr at 110°. A blank with HCl alone was used to correct for the ammonia in the reagents. Cystine was determined as cysteic acid after performic acid oxidation as described by Moore (1963) and tryptophan was

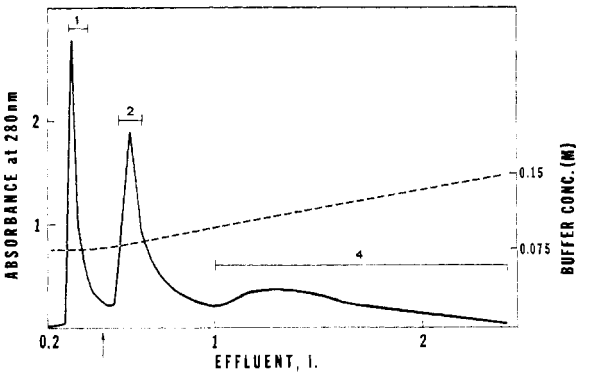

FIGURE 1: Gradient elution of bovine pepsins from hydroxylapatite. Column, 2.0 × 35 cm; sample 800 A_{280} units (540 mg) in 50 ml of starting buffer. Starting buffer: 2 l. of 0.075 M sodium phosphate buffer (pH 5.65). Gradient buffer: 2 l. of 0.15 M sodium phosphate buffer (pH 5.65). The gradient (---) was started at the arrow. The two buffers were in paired interconnected open bottles to give a linear gradient. Flow rate, 15 ml/hr. Fractions, 10 ml. The horizontal bars indicate the portions of the peaks pooled.

measured spectrophotometrically (Edelhoch, 1967) on separate samples. All samples were corrected to dry weight, determined by drying 10-mg samples at 110° *in vacuo*.

Organic Phosphate. The ashing of the protein for total phosphate determination was carried out as described by Meitner and Kassell (1971); the method of Ames (1966) was used for the colorimetric evaluation. Samples were assayed for the presence of any residual inorganic phosphate in the same manner, except for the ashing step, and appropriate small corrections (0.04–0.10 μmole) were made. To determine the recovery of organic phosphate in the presence of protein, 0.01–0.03 μmole of serine *O*-phosphate was added to 0.01 μmole of trypsin (phosphate free by assay). The recovery of the phosphate (82%) was used as a correction in the calculations for organic phosphate. Porcine pepsin was assayed as a control; 0.96 mole/mole of protein was found in agreement with the previous value of 1 mole/mole of protein (Northrop *et al.*, 1948; Perlmann, 1955, 1958). The amounts were computed from an absorbance curve, prepared with standard solutions of KH_2PO_4 and reagent blanks that were subjected to the entire procedure.

Hydrolysis of N-Acetyl-L-phenylalanyl-L-diiodotyrosine. The substrate in amounts to give final concentrations of 3.85–15.5 × 10^{-2} mM was initially dissolved in an amount of NaOH equal to 2 equiv of substrate (Jackson *et al.*, 1965) and brought to volume with 5 mM sodium phosphate buffer containing 3% (v/v) methyl alcohol, at pH 1.35 or 2 (Table II). Aliquots of the solution were incubated at 37.6° with a constant concentration of enzyme (17.6 × 10^{-7} M for bovine and 5.36 × 10^{-7} M for porcine pepsin). The extent of hydrolysis was determined by the liberation of free amino groups. These were measured by the ninhydrin method of Rosen (1957) as modified by Jackson *et al.* (1965). Readings were corrected for ninhydrin-reacting material present at the start by subtracting the absorbance of a solution in which the enzyme was added after adjustment to pH 6. Because of the limited solubility of the substrates, it was not possible to obtain initial rate data at substrate concentrations much greater than K_m. The kinetic parameters K_m, V_{max}, and k_{cat} (molecular activity coefficient) were obtained from a plot of [S]/*v* *vs.* [S] (Dixon and Webb, 1958; Wilkinson, 1961).

Hydrolysis of N-Acetyl-L-phenylalanyl-L-tyrosine. The substrate solutions were prepared at pH 2 and the kinetic studies were carried out in the same manner as above. The substrate concentrations used were 0.6–2.4 mM and the enzyme concentrations were 3.90 × 10^{-5} and 1.60 × 10^{-5} M for the bovine and porcine pepsins, respectively.

TABLE II: Pepsin-Catalyzed Hydrolysis of *N*-Acetyl-L-phenylalanyl-L-diiodotyrosine.[a]

Pepsin	pH	[E] (M × 10^7)	K_m (mM × 10^2)	k_{cat} (min^{-1})
Porcine	1.35	5.36	16.0	9.2
	2.0	5.36	16.9	11.0
Bovine				
1	2.0	17.6	6.4	2.1
2	1.35	17.6	12.2	2.3
4	1.35	17.6	12.6	2.8

[a] All experiments were performed at 37.6° in 3% methanolic 5 mM sodium phosphate buffer. [S] = 3.87 to 15.5 × 10^{-2} mM (Figure 3).

Hydrolysis of Benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-tryptophan Ethyl Ester. The substrate (Inouye *et al.*, 1966) in amounts to give concentrations up to 0.4 mM was initially dissolved in ethyl alcohol so that the final volume of 40 mM citrate buffer (pH 4.0), contained 2% (v/v) ethyl alcohol. The enzyme concentrations were 3.89 × 10^{-6} and 1.16 × 10^{-6} M for bovine and porcine pepsins, respectively.

Results

Amino Acid Composition of Bovine Pepsins. The amino acid analyses of the three bovine pepsins are presented in Table I. The results point to a uniformity in composition in all the pepsins. The presence of only a trace of lysine is notable. A small amount of a basic peptide, formed during the activation, was apparently not removed during the subsequent chromatography. This amount is not enough to affect the whole number values for the other amino acids, as can be seen from the ratios for histidine, arginine, and alanine. When the amino acid residues of the pepsins are subtracted from the residues previously found in bovine pepsinogen and also shown in Table I, the approximate composition of the activation peptides can be derived. They account for about 14% of the pepsinogen molecule.

Organic Phosphate Content of Bovine Pepsins. Bovine pepsins have been reported to differ among themselves in their phosphate content (Meitner and Kassell, 1971). Table I shows that in the present preparations of pepsins obtained by hydroxylapatite chromatography (Figure 1), the organic phosphate contents range from 0.5 to 2.2 moles per mole of protein. These values differ slightly from previous results (0.3–3.1) and are not whole numbers. This may be due in part to inaccuracy in the phosphate determination. Nevertheless, the various fractions are probably still mixtures differing in the number and location of phosphate groups in the molecules.

Kinetic Studies with N-Acetyl-L-phenylalanyl-L-diiodotyrosine. Time course studies at the higher substrate concentrations used showed that the amount of substrate hydrolyzed was nearly a linear function of time until about 35% was hydrolyzed. These results are similar to those of Jackson

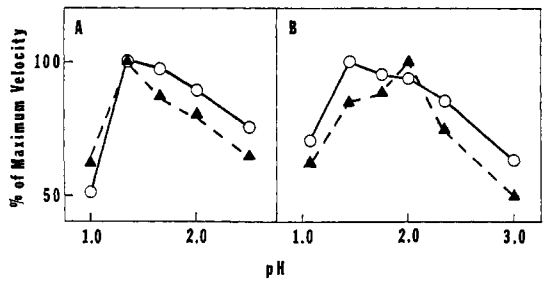

FIGURE 2: Comparative pH–activity profile for the action of bovine (▲) and porcine (○) pepsins on two substrates at 37.6°. (A) *N*-Acetyl-L-phenylalanyl-L-diiodotyrosine at 7.75 × 10^{-2} mM. The enzyme concentration was 17.6 × 10^{-7} and 5.36 × 10^{-7} M for bovine and porcine pepsin, respectively. (B) *N*-Acetyl-L-phenylalanyl-L-tyrosine at 1.8 mM. The enzyme concentration was 2.50 × 10^{-5} and 1.43 × 10^{-5} M for bovine and porcine pepsin, respectively. The buffer used at pH 1 was NaCl–HCl (50 mM) based on sodium ion concentration. The other buffers were 5 mM sodium phosphate, based on sodium ion concentration. All buffers contained 3% methanol.

et al. (1965) for porcine pepsin. The initial velocity was therefore calculated from the amount of hydrolysis (21–27%) that occurred in 8 min.

The pH optimum was determined at a single substrate concentration since the binding of substrates (Denburg *et al.*, 1968) and inhibitors (Knowles *et al.*, 1969) to pepsin does not vary significantly with pH up to pH 3. Plots for the initial velocity (v) *vs.* pH for the hydrolysis of 7.75 × 10^{-2} mM *N*-acetyl-L-phenylalanyl-L-diiodotyrosine were made for all the bovine pepsins and for porcine pepsin. A typical plot is shown in Figure 2A. All the different bovine pepsins have the same pH optimum at 1.35. Porcine pepsin has a broad pH optimum, ranging from 1.35 to 2.0, in good agreement with the results of Baker (1951).

Figure 3 represents a typical plot of [S]/v *vs.* [S] for bovine pepsin fraction 4 and porcine pepsin. The concentrations of the two enzymes were adjusted to give approximately the same percentage of hydrolysis in 8 min. K_m values of 12.6 × 10^{-2} and 16.0 × 10^{-2} mM for bovine and porcine pepsins were obtained by calculation of the x intercepts. These values are not significantly different from each other ($p > 0.05$), as determined by statistical analysis of the difference in altitude of the lines. The molecular activity coefficient (k_{cat}) was calculated from the slope ($1/V_{max}$) and enzyme concentration.

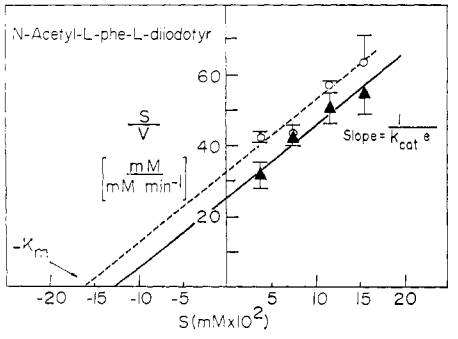

FIGURE 3: Determination of the Michaelis constant, K_m, and the molecular activity coefficient, k_{cat}, from a plot of [S]/v *vs.* [S] for the hydrolysis of *N*-acetyl-L-phenylalanyl-L-diiodotyrosine by bovine (▲) and porcine (○) pepsins. The enzyme concentration was 17.6 × 10^{-7} and 5.36 × 10^{-7} M for bovine and porcine pepsin, respectively. The pH was 1.35.

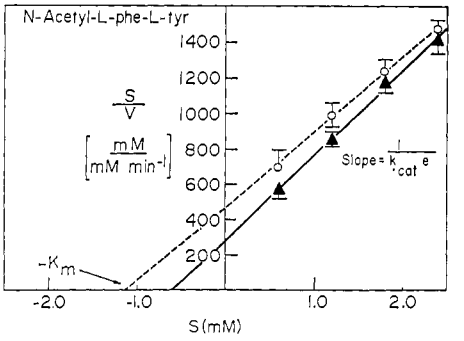

FIGURE 4: Determination of the Michaelis constant, K_m, and the molecular activity coefficient, k_{cat}, from a plot of [S]/v *vs.* [S] for the hydrolysis of *N*-acetyl-L-phenylalanyl-L-tyrosine by bovine and porcine pepsins at pH 2.0. The enzyme concentration for bovine (▲) and porcine (○) pepsins was 3.90 × 10^{-5} and 1.60 × 10^{-5} M, respectively.

Values of 2.8 and 9.2 min^{-1}, respectively, show that bovine pepsin 4 is much less active than porcine pepsin on this substrate. Kinetic parameters obtained in the same manner for the other pepsins are given in Table II. Bovine fraction 1 was studied at pH 2.0 because of some cloudiness at pH 1.35, and for comparative purposes the porcine pepsin experiments were performed at both pH values. All of the pepsins have the same K_m within experimental error ($p > 0.05$). Bovine fractions 2 and 4 show no significant difference in their k_{cat} values ($p > 0.05$).

Kinetic Studies with N-Acetyl-L-phenylalanyl-L-tyrosine. Experiments at the higher substrate concentrations indicated that hydrolysis was a nearly linear function of time until 22% was utilized, in agreement with Jackson *et al.* (1966). An 8-min incubation period at pH 2.0 was chosen and gave 10–17% hydrolysis. A comparison of the pH–activity curves for the action of bovine and porcine pepsin on 1.8 mM substrate (Figure 2B), shows a broad pH range from 1.35 to 2.0 for both species and is similar to that obtained by Baker (1951).

Figure 4 is a typical [S]/v *vs.* [S] plot for bovine fraction 4 and for porcine pepsin at enzyme concentrations adjusted to give similar amounts of hydrolysis. Table III gives the kinetic constants for all the pepsins, calculated in the same way as described for the previous substrate. The K_m values do not differ significantly from each other ($p > 0.05$) except for the difference between bovine pepsin 1 and the other bovine pepsins. Because pepsin 1 was relatively insoluble and caused

TABLE III: Pepsin-Catalyzed Hydrolysis of *N*-Acetyl-L-phenylalanyl-L-tyrosine.[a]

Pepsin	[E] (M × 10^5)	K_m (mM)	k_{cat} (min^{-1} × 10^2)
Porcine	1.6	1.1	15.0
Bovine			
1	3.9	1.8	7.0
2	3.9	0.5	5.4
4	3.9	0.6	5.4

[a] All experiments were performed at 37.6° in 3% methanolic 5 mM sodium phosphate buffer (pH 2.0). [S] = 0.6–2.4 mM (Figure 4).

TABLE IV: Specificity of Pepsins on Several Substrates.

Substrate	Relative Activity			
		Bovine		
	Porcine[a]	1	2	4
Hemoglobin	100	70	60	63
Milk (clotting)	100	92	91	102
N-Ac-L-Phe-L-I_2Tyr	100	19	25	30
N-Ac-L-Phe-L-Tyr	100	47	36	36
Z-L-His-L-Phe-L-TrpOEt	100			10

[a] Prepared by activation of pepsinogen (see text, *Enzymes*).

TABLE V: Amino Acid Compositions of Some Mammalian Pepsins and Gastricsins.

Amino Acid (as Residues/Mole)	Pepsins			Gastricsins	
	Bovine[a]	Porcine[b]	Human[c]	Porcine[d]	Human[e]
Lys	0	1	0	4	0
His	1	1	1	1	1
Arg	3	2	3	4	3
Asp	36	40	40	26	26
Thr	25	25	27	23	21
Ser	44	43	43	32	32
Glu	28	26	31	39	39
Pro	15	16	19	15	17
Gly	33	34	35	31	33
Ala	14	16	18	19	18
Half-Cys	6	6	6	6	6
Val	23	20	27	19	23
Met	3	4	5	4	5
Ile	28	23	25	13	13
Leu	19	28	22	30	25
Tyr	16	16	15	16	17
Phe	14	14	15	19	15
Trp	5	5[e]–6	5	3	4
Total residues	313	321	337	304	298

[a] This paper. [b] Rajagopalan *et al.* (1966). [c] Mills and Tang (1967). [d] Chiang *et al.* (1967). [e] Neradova and Kostka (1970); Vasenev *et al.* (1970).

some problems, this value is less reliable than the others, and the difference may not be real. Comparison of the k_{cat} values shows that the bovine pepsins do not differ from each other ($p > 0.05$). Porcine pepsin has almost three times the activity of the bovine pepsins on this substrate.

Hydrolysis of Benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-tryptophan Ethyl Ester. A time-course study with 0.4 mM substrate and porcine pepsin showed that hydrolysis is a linear function of time up to 20% utilization within a 5-min incubation period. The pH optimum was between 4.0 and 4.5 and is similar to that obtained by Inouye *et al.* (1966). Bovine pepsin was studied at more than three times the enzyme concentration used for porcine pepsin. The bovine pepsin showed so little activity that kinetic constants were not measured.

Digestion of Hemoglobin and Milk Clotting Activity. Table IV summarizes the activities of the different pepsins on various substrates. The bovine pepsins have 60–70% of the activity of porcine pepsin toward hemoglobin substrate, but similar milk clotting activity. Fox (1969) found similar relative milk-clotting and proteolytic activities for bovine and porcine pepsins in determinations carried out at higher pH.

Discussion

With synthetic substrates, bovine pepsin is a far less efficient hydrolyzing enzyme than porcine pepsin (difference in k_{cat}), although it is able to bind the substrates just as well (no differences in K_m). With hemoglobin as substrate, bovine pepsin is also less efficient, but the difference is smaller (Table IV). Chicken pepsins resemble porcine pepsin in activity on hemoglobin substrate, but have much less milk clotting activity and almost no activity on three synthetic substrates (Levchuk and Orekhovich, 1963; Donta and Van Vunakis, 1970). The difficulty of making comparisons of kinetic data obtained in different laboratories has been discussed by Jackson *et al.* (1969). Methanol is known to increase K_m (Tang, 1965) and with some substrates to decrease k_{cat} (Zeffren and Kaiser, 1967). We found it necessary to use methanol, but kept the concentration down to 3%. Considering the differences in methanol concentration, as well as in pepsin preparations and temperature, the present data for porcine pepsin are in reasonable agreement with previous studies with the same substrates (Jackson *et al.*, 1965, 1966; Silver *et al.*, 1965).

The kinetic studies presented have shown that the differences in phosphate found in the bovine pepsins do not alter either the binding of substrates or the efficiency of their hydrolysis. This is in agreement with some of the studies on porcine pepsin; Perlmann (1958) and Clement *et al.* (1968) observed that the single phosphate could be removed without significant effect on the activity. Lee and Ryle (1967a), however, found differences in milk clotting and gelatin-digesting action.

The composition studies of Table I show that the bovine pepsins are identical or nearly identical with each other in amino acid content. The differences in amide groups are not significant, considering the standard deviations and the large number of amide groups. These results are in agreement with our previous findings of identity in composition of the pepsinogens from which these pepsins were derived (Meitner and Kassell, 1971). This previous work also showed that bovine pepsins contain no carbohydrate, and that all of the pepsins have amino-terminal valine and carboxyl-terminal alanine. Thus, contrary to other species, zymogen synthesis in the cow involves only a single protein, which is then modified by the addition of phosphate groups.

Comparison of the composition of bovine pepsin to other mammalian gastric enzymes (Table V) shows that the similarities in general composition are very marked: the small number of basic residues, the very large number of acidic amino acids and serine and almost constant amounts of cystine and aromatic amino acids. There are, however, numerous substitutions in individual amino acid residues. Bovine pepsin has no lysine; in this respect it resembles human pepsin and gastricsin, but differs from the porcine enzymes. Valine, isoleucine, and leucine are quite variable among these enzymes. We predict that some of the amino acid substitutions that dis-

tinguish porcine and bovine pepsins are close to the catalytic site in space, to account for the large quantitative differences in activity observed.

The mammalian pepsins, when compared to chicken pepsins (Bohak, 1969; Donta and Van Vunakis, 1970), have much smaller contents of basic amino acids and methionine.

Determination of the exact number of tryptophan residues is difficult. By the spectrophotometric method of Edelhoch (1967), using both Worthington and chromatographed samples of porcine pepsin, values ranging from 5.2 to 6.1 residues have been obtained, with an average of about 5.5 (*cf.* Rajagopalan *et al.*, 1966; Neradova and Kostka, unpublished data 1969 (as quoted by Kostka *et al.* (1970)); Vasenev *et al.*, 1970). The values for the bovine pepsins are close to 5 residues (Table I). At this level of tryptophan content, we do not think the method is more accurate than ± 0.5 residue.

The determination of the approximate size and composition of the activation peptides by difference (Table I) defines the process of activation as quite similar to that for porcine pepsin. Both zymogens lose peptides from their amino terminus (*cf.* Herriott, 1962; Meitner and Kassell, 1971). The two sets of peptides are of similar total amino acid composition (Ong and Perlmann, 1968, and Table I), and both are highly basic. The sequence of the porcine activation peptides is known (Ong and Perlmann, 1968). The fact that one of them is a pepsin inhibitor (Herriott, 1941; Van Vunakis and Herriott, 1956) arouses interest in the bovine activation peptides also, and they are under investigation.

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